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# Ultrastructure of Spermatozoa and Spermatogenesis in Nepomorpha (Insecta: Heteroptera) with Special Reference to Phylogeny

Young Hwan Lee[1] and Chang Eon Lee[2]

[1]*Department of Biology Education, College of Education, Taegu University,*
*Kyungsan 713-714, and* [2]*Department of Biology, College of Natural*
*Sciences, Kyungpook National University, Taegu 702-701, Korea*

**ABSTRACT**—Ultrastructural study of spermatozoa was made for five species and four genera in two families of Nepomorpha, Belostomatidae and Nepidae (Insecta: Heteroptera). The following five synapomorphic relationships were recognized for the fine structures of spermatozoa: 1) Nepomorpha have acrosome displaced to a lateral position with respect to the nucleus; 2) the centriole adjunct is well developed in mature spermatozoa; 3) each of the two mitochondrial derivatives contains two or three crystalline bodies; 4) the derivatives are linked to the axoneme by two bridges at the level of doublets 1 and 5; and 5) accessory bodies are absent in this group. A brief comments on the phylogenetic relationships among three families (Belostomatidae, Nepidae and Notonectidae) of Nepomorpha is given.

## INTRODUCTION

The aquatic heteropteran group Nepomorpha (=Cryptocerata or Hydrocorisa; Insecta) recorded in Korea comprise 32 species, belonging to 16 genera of 8 families. Lee [1] attempted the reconstruction of Nepomorpha phylogeny based on adult characters, in particular fine structures of genitalia.

Though comparative spermatology seems to provide useful information for heteropteran systematics, only few papers have been published on Nepomorpha. Itaya *et al.* [2] described fine structures of the later stages of spermiogenesis in *Leptocoris trivittatus* Provancher (Corixidae), but did not refer to mature spermatozoon. Descriptions of the sperm in *Nepa rubra* L. (Nepidae) were given by Werner [3].

In Nepomorpha as a whole, a few works on mature spermatozoa of the following species have been made: *Diplonychus esakii* Miyamoto et Lee [4] (Belostomatidae), *Ranatra unicolor* Scott [5] and *Laccotrephes japonensis* Scott [6] (Nepidae) and *Notonecta glauca* L. [3, 7–9] (Notonectidae). In Notonectidae no information on the centriole adjunct is available. Recently Lee and Lee [5] reported the centriole adjunct of mature spermatozoa of *Ranatra unicolor* Scott.

In this paper we report and compare some ultrastructures of the spermatozoa and spermatogenesis in Nepomorpha.

## MATERIALS AND METHODS

The following five species of Nepomorpha were examined in this study: Belostomatidae—*Muljarus japonicus, Diplonychus esakii;* Nepidae—*Ranatra unicolor, R. chinensis, Laccotrephes japonensis*. They were all collected near Taegu, Korea.

For transmission electron microscopy, testes and seminal vesicles of the adult males were fixed in 2.5% glutaraldehyde in a 0.1 M cacodylate buffer, rinsed in 0.1 M cacodylate buffer, pH 7.2, postfixed in 1% osmium tetroxide for 40 minutes, dehydrated in ethanol, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a JEOL 100 S and a Hitachi H-600.

For light microscopy, a drop of suspension of spermatozoa obtained by squeezing the seminal vesicle in cacodylate buffer was smeared on a slide, covered with a slide cover and photographed with

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Olympus multipurpose microscope.

## RESULTS AND DISCUSSIONS

### 1. *Morphology of the spermatozoa and spermatogenesis in Nepomorpha*

Spermatozoa and the structural changes that occur during spermatogenesis in the five species of Nepomorpha have been examined. The spermatozoa of the Nepomorpha vary in length in different species, from 210 μm or less to 16.5 mm or more. The head is from 20 μm to 1.16 mm long (Table 1).

### 1.1 Belostomatidae

*Muljarus japonicus* has spermatozoa measuring

TABLE 1. Summary of the morphology of the sperms and spermatogenic cells in 6 species of Nepomorpha

| B | | Ne | | | N | Character state | | Character |
|---|---|---|---|---|---|---|---|---|
| Mj | De | Ru | Rc | Lj | Ng | | | |
| | ▬ | | ▬ | | | 200–220 μm or less | | Total length of the sperm |
| | | ▬ | | | | 221–240 μm | | |
| ▬ | | | | | | 241–260 μm | | |
| | | | | ▬ | | 350–370 μm | | |
| | | | | | ▬ | 15 mm or more | | |
| ▬ | | ▬ | ▬ | | | 30–40 μm or less | | Head length |
| | | | | ▬ | | 41–50 μm | | |
| | ▬ | | | | | 61–70 μm | | |
| | | | | | ▬ | 1 mm or more | | |
| ▬ | ▬ | ▬ | ▬ | ▬ | ▬ | Lateral disposition | | Acrosome |
| ▬ | ▬ | | | | | 0.1–10 $\mu$m$^3$ | volume | |
| | | ▬ | ▬ | | | 11–50 $\mu$m$^3$ | | |
| | | | | ▬ | | 51–100 $\mu$m$^3$ | | |
| | | | | | ▬ | 10,000 $\mu$m$^3$ or more | | |
| ▬ | | ▬ | ▬ | ▬ | ▬ | Compact | | Texture of acrosome |
| | ▬ | | | | | Not compact | | |
| ▬ | ▬ | | | | ▬ | Absent | | Microtubules within acrosome |
| | | ▬ | ▬ | ▬ | | Present | | |
| ▬ | ▬ | ▬ | ▬ | ▬ | ▬ | Tubular type | | Subacrosomal lumen |
| | | ▬ | ▬ | ▬ | ▬ | Circular type | | Nuclear section |
| ▬ | ▬ | | | | | Kidney type | | |
| ▬ | ▬ | ▬ | ▬ | ▬ | ND | Long | | Centriole adjunct |
| ▬ | ▬ | ▬ | ▬ | ▬ | | Abundant | | |
| ▬ | ▬ | ▬ | ▬ | ▬ | ▬ | Axoneme (9+9+2) | | Tail |
| ▬ | ▬ | ▬ | ▬ | ▬ | ▬ | Bridges between A and MD | | |
| ▬ | ▬ | ▬ | ▬ | ▬ | ▬ | MD crystallization | | |
| ▬ | ▬ | ▬ | ▬ | ▬ | | Equal | | MD symmetry |
| | | | | | ▬ | Not equal | | |

B: Belostomatidae; Ne: Nepidae; N: Notonectidae. Mj: *Muljarus japonicus*; De: *Diplonychus esakii*; Ru: *Ranatra unicolor*; Rc: *Ranatra chinensis*; Lj: *Loccotrephes japonensis*; Ng: *Notonecta glauca*; ND: no data.

about 260 μm in total length (Fig. 1), 0.4 μm in diameter at the head and 0.6 μm at the tail, the head being 30–35 μm long. *D. esakii* has spermatozoa measuring 210 μm in total length, the head being 65 μm long (Fig. 10). Spermatozoa of *D. esakii* are longer and the heads are shorter than those of *M. japonicus*.

The acrosome is displaced to a lateral position with respect to the nucleus in the family (Fig. 2) as well as in the other examined groups of Nepomorpha (Figs. 11, 16, 26, 30). The family has an acrosome of very small size, approximately 1.4 $\mu m^3$ in volume in the early spermatid of *Muljarus japonicus* (Fig. 6) and 0.9 $\mu m^3$ in *Diplonychus esakii*. The acrosome of *M. japonicus* is composed of an electron-dense material during the spermatid differentiation (Fig. 6), whereas that of *D. esakii* is very low in electron density (Fig. 11).

The nucleus of the sperm shows kidney-shape in its cross section during the differentiation of the spermatid (Figs. 3, 13). The morphology of the nucleus is distinctly different when compared with those of other families of Nepomorpha (Table 1). The nuclear membrane holds in its posterior concavity the centriole adjunct in early spermatid and they form the nucleus-centriole adjunct complex (Fig. 5).

The spermatid tail elongates tangentially at the proximal portion of the nucleus (Fig. 4). The centriole adjunct is abundant and displaced to a lateral nucleus in mature spermatozoa (Figs. 8, 12). The sperm of this family has a "9+9+2" axonemal pattern (Figs. 9, 14). A sperm bundle contains 1024 spermatozoa (Fig. 7), the mitosis occurring eight times at the spermatogonia.

## 1.2 Nepidae

*Ranatra unicolor* spematozoa are about 225 μm long (Fig. 15); the head about 20 μm long and 0.5 μm wide at the base and 0.4 μm wide at the tip. The mature acrosome is approximately 5.83 μm long and 0.33 μm thick (Fig. 16). The acrosome contains 220–250 Å wide longitudinal tubules through its length (Fig. 17) [5]. The acrosome of the early spermatid is approximately 30 $\mu m^3$ in volume (Fig. 18).

*R. chinensis* spermatozoa are about 220 μm long (Fig. 24); the head about 20 μm long and 0.8 μm wide at the base, 0.5 μm wide at the tip. The early acrosome of approximately 20 $\mu m^3$ in volume is smaller than that of *R. unicolor*.

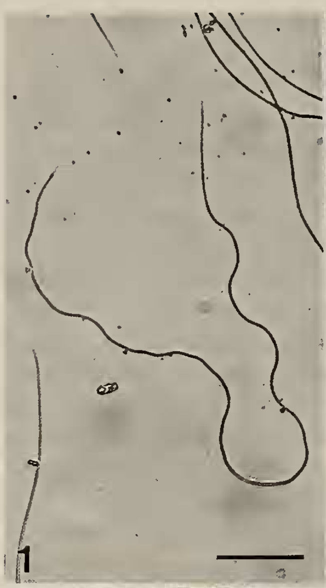
The filiform spermatozoa of *Laccotrephes japonensis* are about 370 μm long (Fig. 29); the head about 45 μm long and 0.33 μm wide at the tip and 0.5 μm wide at the base. A sperm bundle of *L. japonensis* contains 512 spermatozoa (Fig. 35). The acrosome forms a spear with the hooked tip. The posterior surface of the acrosome invaginates to the one side of the nucleus (Fig. 30). The acrosome of the early spermatid is approximately 2.5 μm in diameter and 65.4 $\mu m^3$ in volume (Fig. 36), which is the largest one of the Nepidae.

The acrosome of Nepidae is much longer and larger than that of Belostomatidae and also forms an apical cap, extending on one side along the anterior nucleus (Figs. 16, 30). The family has a prominent acrosome which contains tightly packed tubules (Fig. 17). An acrosome with tubules has been described from Gerromorpha but not from acrosomes of other animals [10, 11]. Dallai and Afzelius [12] have proposed that this type of acrosome represents an adaptation to life in fresh water. The subacrosomal space forms an elongated tube extending to its anterior end through the acrosome.

The nucleus is a cylindrical rod with highly condensed chromatin, but apically is narrowed and surrounds the acrosome which is in cross section embedded in an opened thick ring of the nucleus (Figs. 19, 26, 31). The anterior and posterior nuclear cross-sections contain the acrosome and the centriole adjunct of crescent-shape respectively (Figs. 21, 26, 27, 34).

The family has well-developed centriole adjunct of very elongated and electron dense structure in mature sperm (Figs. 20, 21, 25, 27, 33, 34). The centriole adjunct of *R. unicolor* lengthens to at least 10 μm more in nearly mature spermatozoa (Fig. 20). It extends anteriorly lateral to the basal nucleus and surrounds posteriorly the outside of the mitochondrial derivatives and flagellum.

The lateral position of the centriole adjunct is very characteristic in Nepidae as well as Belostomatidae. In particular two families have abundant centriole adjunct displaced to a lateral nucleus in mature spermatozoa (Figs. 5, 8, 12, 20, 25, 33).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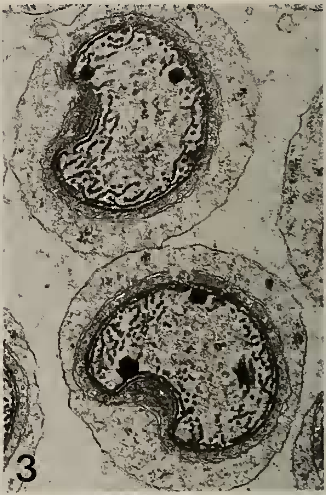
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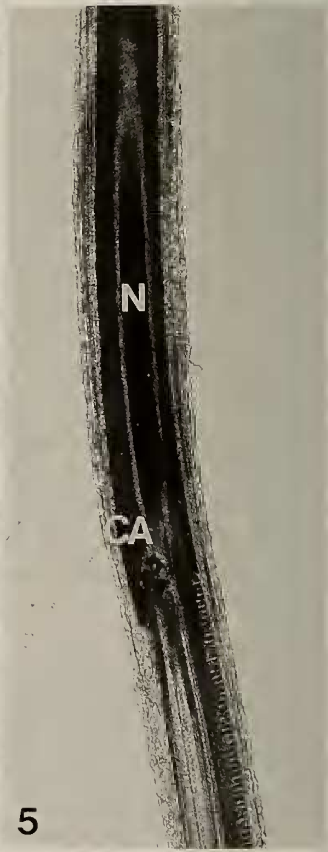
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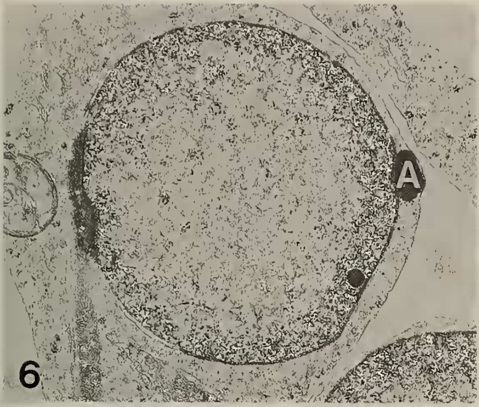
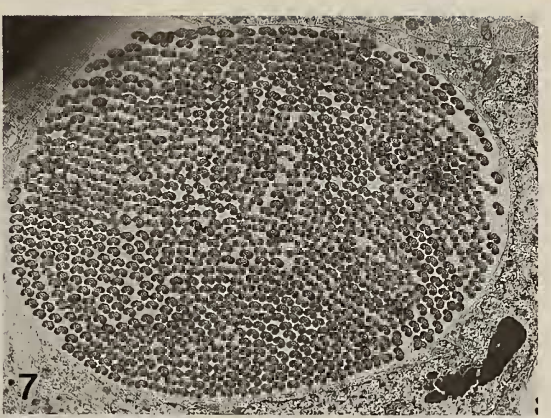
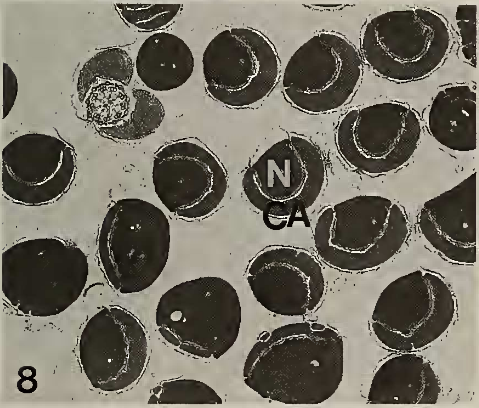
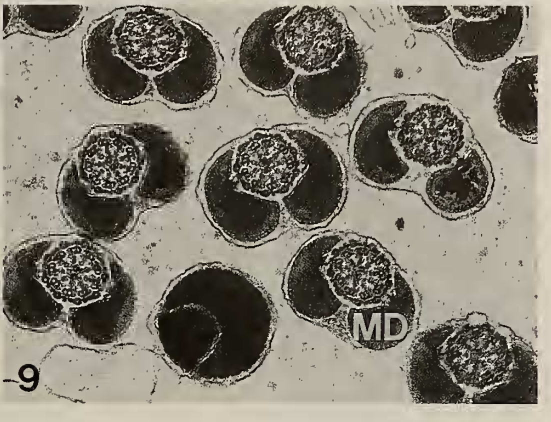








FIGS. 1–9. *Muljarus japonicus* (Belostomatidae).

Abbreviations in these and subsequent figures: A=acrosome; Ax=axoneme; N=nucleus; CA=centriole adjunct; MD=mitochondrial derivatives

FIG. 1. Mature spermatozoa. Scale bar; 25 μm.

FIG. 2. Acrosome displaced to a lateral nucleus. ×6,000.

FIG. 3. Cross section of nucleus in spermatid. ×18,000.

FIG. 4. Tail elongating tangentially at the proximal portion of the nucleus. ×30,000.

FIG. 5. Longitudinal section of head-tail junction. ×15,000.

FIG. 6. Acrosome of the early spermatid. ×10,000.

FIG. 7. Cross section of a sperm bundle. ×2,300.

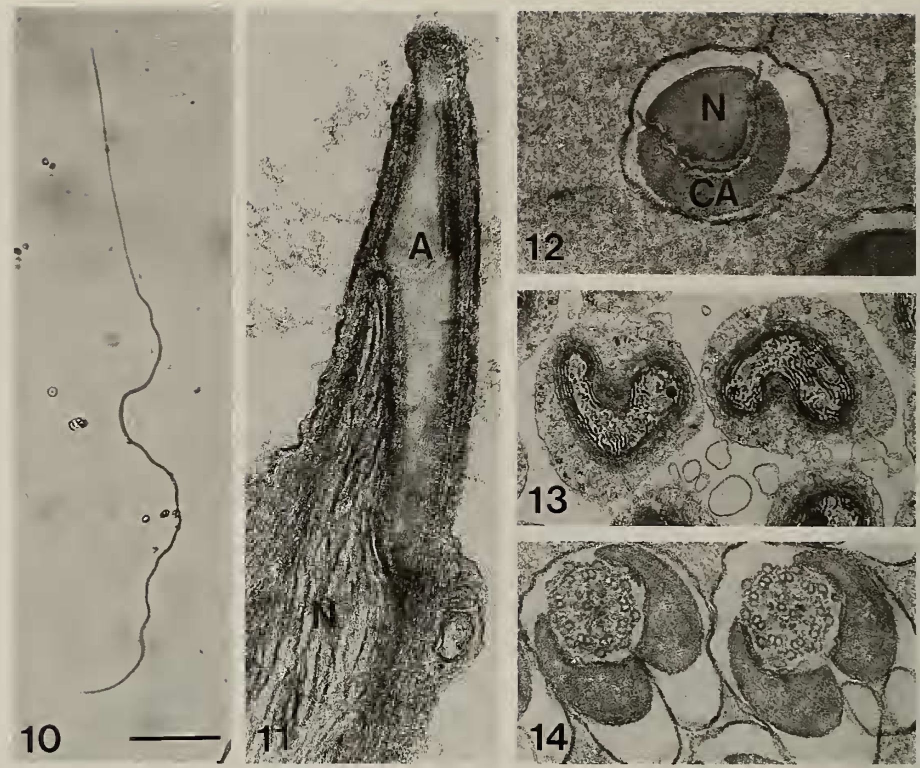
FIG. 8. Cross section of nucleus-centriole adjunct complex. ×20,000.

FIG. 9. Cross section of tail. ×30,000.

The nucleus-centriole adjunct complex follows two mitochondrial derivatives which surround the axoneme. The microtubules are ordered in hexagonal and clover arrangement around the nucleus-centriole adjunct complex during the spermatid differentiation (Figs. 23, 37).

The tail appears symmetrical. It is made up of an ordinary axoneme and two mitochondrial derivatives of equal size. These components are arranged throughout the length of the tail. The mitochondrial derivatives are the bilateral symmetry of the axoneme (Fig. 22). They surround most of the axoneme in Nepidae (Figs. 22, 28, 32). The horizontally oriented cristae exhibit a very regular periodicity of approximately 55–57 nm in *R. unicolor* (Fig. 20), 47 nm in *R. chinensis* (Fig. 25) and 41 nm in *Laccotrephes* (Fig. 33).

The axoneme belongs to the basic 9+9+2 pattern, having evident radial links with prominent heads, evident dynein arms on the doublets, and a



Figs. 10–14. *Diplonychus esakii* (Belostomatidae).
Fig. 10. Mature spermatozoa. Scale bar; 25 μm.
Fig. 11. Acrosome showing very low electron density in spermatid. ×42,000.
Fig. 12. Cross section of nucleus-centriole adjunct complex. ×69,000.
Fig. 13. Cross section of nucleus in spermatid. ×12,000.
Fig. 14. Cross section of tail. ×49,000.

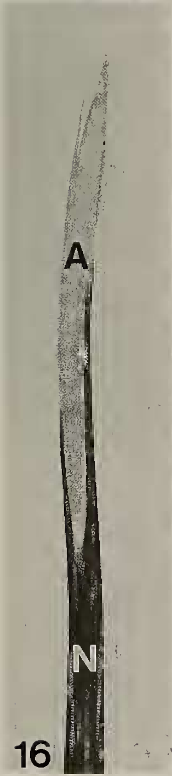
layer of 9 accessory tubules. The diameter of the crystallization of the mitochondrial derivatives may measures more or less half of the section in the all examined group (Figs. 9, 22, 28, 32). The axoneme is flanked by two mitochondrial derivatives but lacks accessory bodies, such as are found in spermatozoa from Homoptera and many related groups of insects [13].

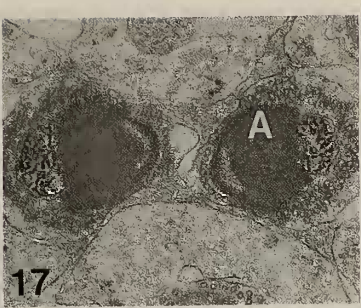
Three further features are also characteristic in Nepomorpha as follows: (1) the presence of two bridges that join the mitochondrial derivatives to the axoneme at the level of the microtubular doublets nos. 1 and 5; (2) the occurrence of two or three crystalline bodies in the mitochondrial derivatives, rather than a single one as is common in pterygotes; (3) the abundant centriole adjunct in mature sperm. The third feature seems to be unique to spermatozoa of Nepomorpha, such as is found in spermatid of Pentatomidae but has not been observed in mature sperm [14].

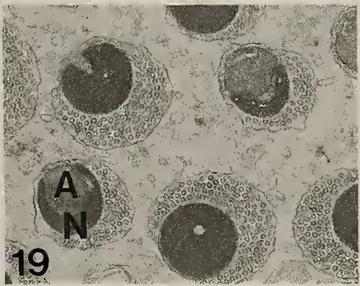
### 1.3 Notonectidae

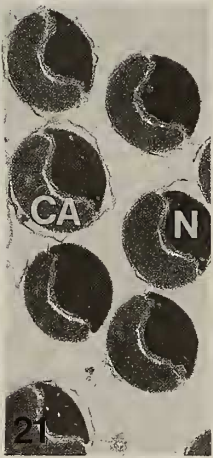
Estimations of the sperm length have ranged between 14 mm for *Notonecta maculata* [15] to 16.5 mm for *N. glauca* [16]. They are the longest one among the animal kindgom. *N. glauca* described previously [3, 7–9] are reanalysed in the light of phylogeny of Nepomorpha.

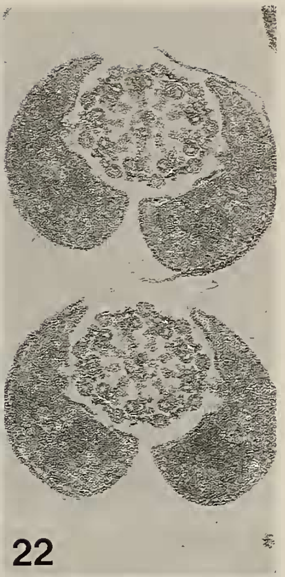
Two special structures appear in connection with the nucleus during spermatogenesis in *Notonecta* species. These are pseudochromosomes in spermatocytes and nuclear calottes in mid spermatids.



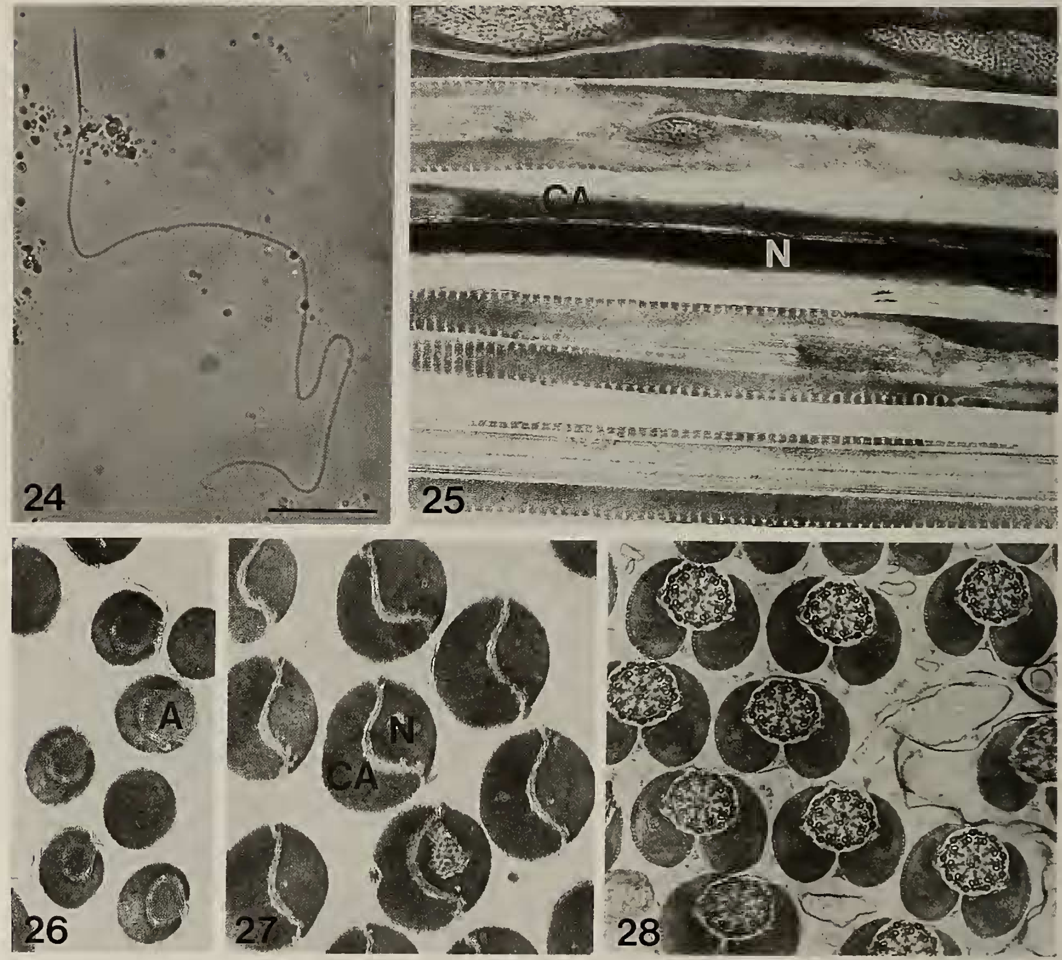








FIGS. 15–23. *Ranatra unicolor* (Nepidae).
FIG. 15. Mature spermatozoon. Scale bar; 50 μm.
FIG. 16. Longitudinal section of acrosome. ×12,000.
FIG. 17. Cross section of acrosome containing tightly packed tubules. ×30,000.
FIG. 18. Acrosome of the early spermatid. ×12,000.
FIG. 19. Cross section of the head. ×24,000.
FIG. 20. Longitudinal section of centriole adjunct. ×8,000.
FIG. 21. Cross section of nucleus-centriole adjunct complex. ×24,000.
FIG. 22. Cross section of tail. ×60,000.
FIG. 23. Cross section of microtubules between the nucleus and a membranous sleeve. ×30,000.

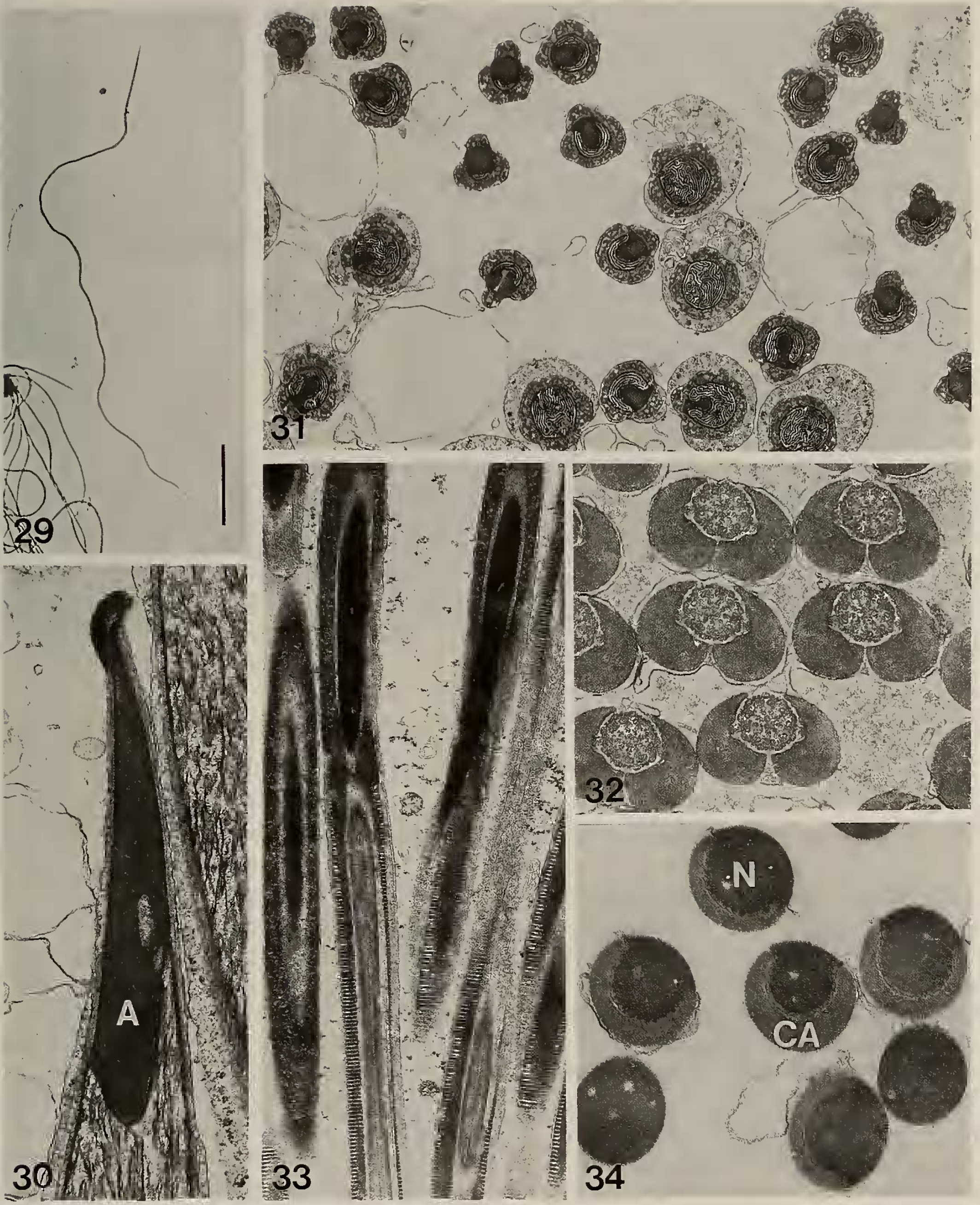


FIGS. 24–28. *Ranatra chinensis* (Nepidae).
FIG. 24. Mature spermatozoon. Scale bar; 25 μm.
FIG. 25. Longitudinal section of sperms showing nucleus-centriole adjunct complex and tail. ×30,000.
FIG. 26. Cross section of head. ×32,000.
FIG. 27. Cross section of nucleus-centriole adjunct complex. ×32,000.
FIG. 28. Cross section of tail. ×32,000.

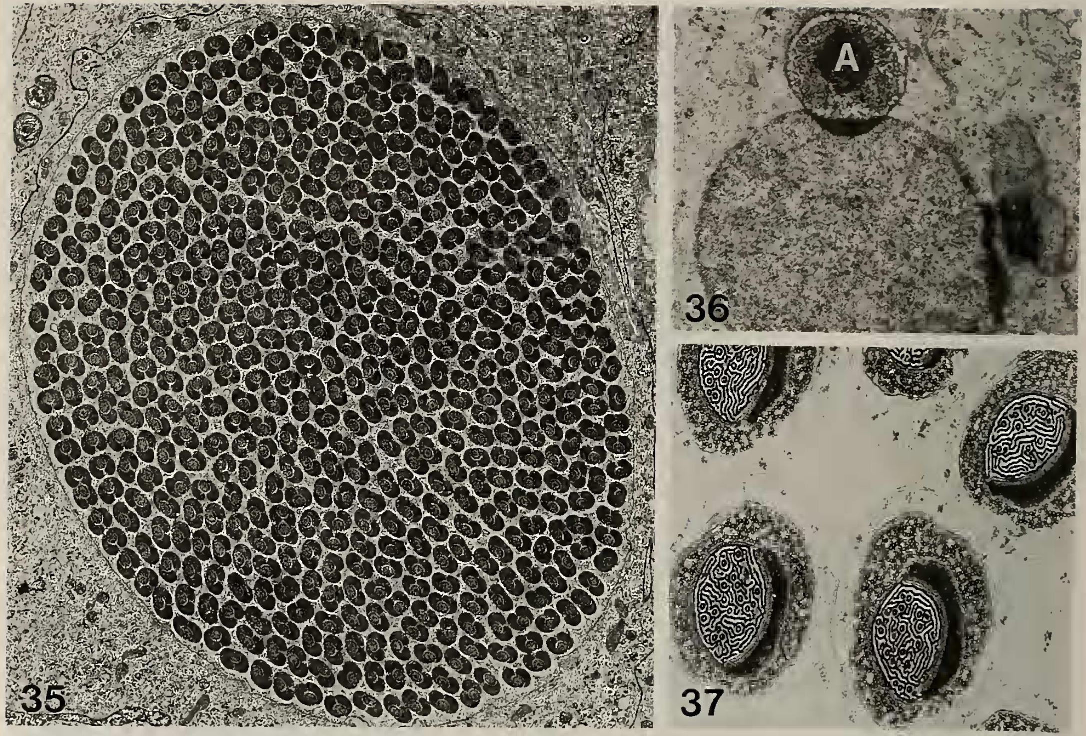
In spermatocytes the membrane system consists of a shell of two or three parallel cisterns enclosing the whole nucleus at a certain distance from its envelope. In young spermatids remnants of the former shell change to spherical calottes and become attached to the nucleus. After separation from the nucleus it ultimately disintegrates to whirls of cisterns still visible in late spermatids [8].

The acrosome grows to a sphere measuring about 20 μm in diameter and 33493.33 $\mu m^3$ in volume. During elongation the acrosome attains spindle shape, the lateral process gradually becoming its anterior part [7]. The acrosome of the backswimmer resembles somewhat that of two other hemipterans, *Nepa rubra* L. [17] and *Gerris remigis* [10]. At its posterior end the acrosome has a lateral groove. It appears empty at some levels but contains the nucleus and the centriole at a more posterior level.

The axoneme is of a common type among insects with a 9+9+2 pattern, and it is unusual only by its great length. The larger mitochondrion occupies



Figs. 29–34. *Laccotrephes japonensis* (Nepidae).
Fig. 29. Mature spermatozoon. Scale bar; 50 $\mu$m.
Fig. 30. Longitudinal section of head. ×16,000.
Fig. 31. Cross section of head. ×9,000.
Fig. 32. Cross section of tail. ×30,000.
Fig. 33. Longitudinal section of sperms showing nucleus-centriole adjunct complex and tail. ×12,000.
Fig. 34. Cross section of nucleus-centriole adjunct complex. ×30,000.



Figs. 35–37. *Laccotrephes japonensis* (Nepidae).
Fig. 35. Cross section of a sperm bundle. ×4,000.
Fig. 36. Acrosome of the early spermatid. ×10,000.
Fig. 37. Cross section of microtubules between the nucleus-centriole adjunct complex and a membranous sleeve. ×18,000.

70–80% of the tail cross-section, the smaller one 10–15%. The two mitochondria are completely embracing the axoneme. The mitochondria have regularly arranged cristae. The three paracrystalline bodies can be recognized in both the small and the large mitochondrion. The shapes of the bodies differ in the two mitochondria. The paracrystalline bodies occupy most of the mitochondrial volume [7].

### 2. *Phylogenetic relationships among three families in Nepomorpha based on the spermatozoal ultrastructures*

In an investigation of spermatozoal ultrastructure from six species of Nepomorpha, the position of the acrosome and centriole adjunct appears to be specific for Nepomorpha and the centriole adjunct is well developed in mature spermatozoa.

*Diplonychus esakii* and *Muljarus japonicus* are easily distinguished by the following characteristics: (1) the total length of the sperm of *M. japonicus* is longer than that of *D. esakii*, on the other hand the head length is shorter than that in *D. esakii*; and (2) the acrosome has low electron density in the spermatid of *D. esakii*, while *M. japonicus* has a dense electron acrosome through the sperm differentiation. The differences between the two species are evident and they are not closely related, although they are grouped to the same genus, until Lee [1] established a new genus *Muljarus* for the species of *japonicus* groups.

Two species of *Ranatra* are closely related by the characteristics showed in Table 1. *Laccotrephes* has many resemblances to *Ranatra* in nucleus, acrosome and centriole adjunct and two genera should be related to each other closely grouped.

Both Belostomatidae and Nepidae are closely related based on the ultrastructures of acrosome, subacrosomal lumen, centriole adjunct and mitochondrial derivatives, though they are different in the morphology of the nucleus during the differentiation and in the microtubules within the acrosome. On the contrary, *Notonecta* are characterized by the ultrastructure of mitochondrial derivatives with two different sizes and the giant sperm and the acrosome.

Miyamoto [18] considered that the Nepidae and Belostomatidae are of the same evolutional directions in the structures of the alimentary organs. Cobben [19] lined Nepidae with Belostomatidae based on the distribution of the larval abdominal glands and the structures of stylets. Lee [1] also grouped the Belostomatidae with Nepidae based on the genital structures and subdivided Nepomorpha into four family groups, Belostomatidae and Nepidae; Notonectidae, Pleidae and Helotrepidae; Aphelocheiridae, Naucoridae, Gelastocoridae and Ochteridae; and Corixidae. Moreover he suggested that they do not show any phylogenic trend in genital structures of the four groups and the different characters might have been derived from the polyphyletic terrestrial origins.

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